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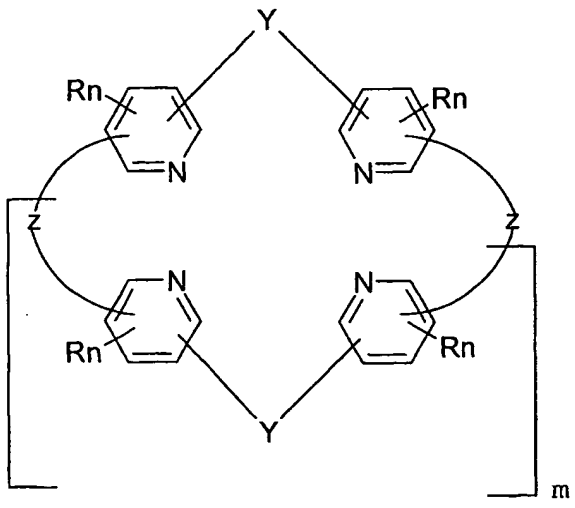
(54) Title: NITROGENEOUS POLYCYCLIC DERIVATIVES USEFUL AS CHELATORS OF METAL IONS AND THEIR APPLICATIONS



(I)

(57) Abstract: The invention relates to the use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases, said derivatives having formula : (I) wherein Rn is R1, R2, R3 and R4, identical or different and represent H or one or several radicals selected in the group comprising -OH, alkyl, -O-alkyl, -NH?2#191, -NH-alkyl, -N (R5, R6), the alkyl being a C1-C6 alkyl, or an halogen, - Y forms a phenyl with both pyridines, optionally ortho-substituted by R5, or ortho-disubstituted by R5 and R6, said substitutuents, identical or different, being selected amongst alkyl, -O-alkyl, -NH?2#191, -NH-alkyl, -N (R5, R6), the alkyl being a C1-C6 alkyl, or an halogen, or represents -(CH?2#191)?m1#191-W-(CH?2#191)?m1#191, with M1 and M2 being 0, 1 or 2, and W being a group -CH?2#191-, -CH-(R7), 0, or N (R8, R9), R7, R8 and R9, identical or different, being a C1-C3 alkyl radical, or H, - Z is -A-(CH?2#191)?m#191-U-(CH?2#191)?n#191-A-, with A = O or N, and U = -(CH?2#191)?n1#191-, -N(R1, R2), -COOH, -OH, with n is 2 to 6, and n1 is 0 or 1, and the complexes thereof with transition metals.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

"Nitrogeneous polycyclic derivatives useful as chelators of metal ions and their applications"

The invention relates to the use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases. Said derivatives are useful as ligands to form complexes with transition metals, and the invention also relates to the use of such derivatives containing ligands as active principles.

Many studies have recently shown the major role of metal ions (copper, zinc, iron, ...) in modification of the folding or the aggregation of proteins, leading then to serious pathologies. Several neurodegenerative diseases (Alzheimer's disease, Parkinson and Huntington diseases, spongiform encephalopathies, ...) involve these disastrous non-desired interactions between metal ions and proteins.

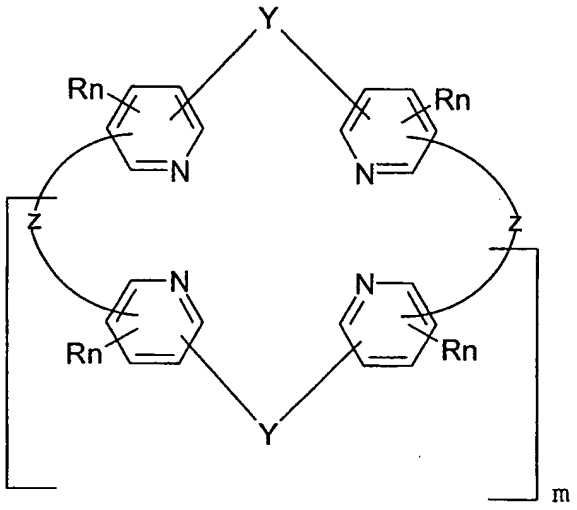
In the case of Alzheimer disease, the pathology is associated with the aggregation of □-type amyloid peptides in the brain, leading to the formation of amyloid plaques. The accumulation of redox active metal ions in these amyloid plaques is deemed to be responsible for oxidative stress inducing neuronal lesions in the brain which result in irreversible loss of intellectual faculties.

The use of a ligand of metal ions like Clioquinol led to improvements in Alzheimer's disease indicating that therapeutic approaches are possible with metal ion chelators in neurodegeneratives diseases.

Recent works of the inventors on phenanthroline derivatives ("Phen" will be used to designate 1,10-phenanthroline) has demonstrated the benefit of complexing copper with two phenanthroline ligands connected to each other. It was therefore decided to prepare new cyclic uncharged ligands called "Cyclo-Phen", small and sufficiently hydrophobic to be able to cross the barriers (first the

intestinal barrier and then the blood brain barrier to go to coordinate the metal ions (copper in preference) which are present in excess in the pathogen proteins.

The invention thus relates to the use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases, said derivatives having formula (I)



wherein

- Rn is anyone of R1, R2, R3 and R4, which are identical or different and represent H or represent one or several radicals and are selected in the group comprising -OH, an alkyl radical, -O-alkyl group, $-NH_2$,-NH-alkyl, -N (R5, R6), the alkyl being in said radical or groups a C1-C6 alkyl, or an halogen selected between the group consisting of F, Cl, Br,

- Y

- forms a phenyl group with both pyridines, optionally ortho-substituted by a substituent R5, or ortho-disubstituted by R5 and R6, said substituents being identical or different, and selected in the group comprising an alkyl radical, -O-alkyl group, $-NH_2$,-NH-alkyl, -N (R5, R6), the alkyl being in said radical or groups a C1-C6 alkyl, or an halogen selected between the group consisting of F, Cl, Br, or

- represents a group - $(CH_2)_{m1}$-W - $(CH_2)_{m1}$-, with m1 and m2 being 0, 1 or 2, and W being a group $-CH_2-$, -CH (R7), O, or N (R8, R9), R7, R8 and R9, identical or different, being a C1-C3 alkyl radical, or H,

- Z is a linking arm of formula - A- $(CH_2)_n$-U- $(CH_2)_n$-A-,

- A being O or NH, and
- U being selected in the group comprising - $(CH_2)_{n1}$-, - N (R1,R2), -COOH, -OH,

with n being a number from 2 to 6, preferably from 2 to 4, and n1 being 0 or 1,

and the complexes thereof with transition metals, particularly with copper, zinc or iron.

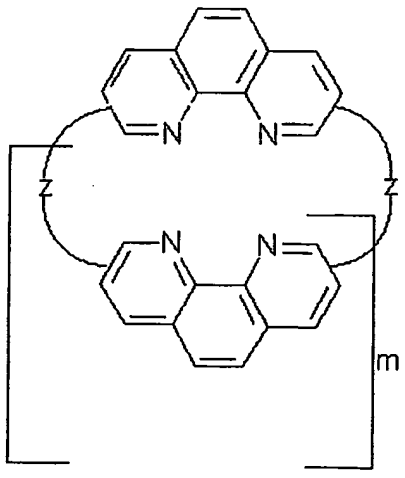
According to an embodiment of the invention, said derivatives include 2 cyclic moieties.

According to another embodiment of the invention, said derivatives include 3 cyclic moieties.

According to still another embodiment, said derivatives include 4 cyclic moieties.

Preferably, the cyclic moieties consist of Phen moieties.

The invention particularly relates to the use of polycyclic Phen derivatives having formula (II)

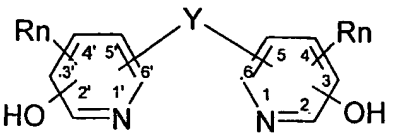


The invention particularly relates to the use of derivatives having 2, 3 or 4 Phen moieties.

The invention also relates to a method for the preparation of said derivatives.

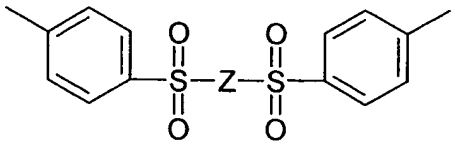
The method of the invention comprises reacting

- a dihydroxy bipyridine derivative of formula (III)



with

- a ditosyl derivative of formula (IV)



wherein Rn, Y and Z are as above defined.

The reaction is carried out with high dilution conditions to limit oligomerizations.

The precursor of formula (III) is preferably used at concentrations of 0.1 to 20 mM in a polar solvent, such as DMSO.

In order to avoid β-elimination reactions, a weak base like cesium carbonate is used.

The derivatives of the invention have a low molecular weight (MW of 504 for the cyclic bi-Phen) and are poorly charged. Therefore they are able to cross the blood brain barrier in both directions (the metal ions present in excess in the pathogen proteins have to be chelated and the resulting complex has to be exported towards the blood circulation conducting to its ultimate excretion),

Their structure can be altered to adjust the chelation selectivity in order to target certain metal ions.

It results from the pharmacological studies carried out with said derivatives that they have new activity spectrum and

are particularly appropriate for the treatment of neurodegenerative diseases as above mentioned.

The invention relates to the use of said derivatives for preparing drugs for treating degenerative diseases comprising Alzheimer, Parkinson, Huntington diseases.

Said drugs comprise an effective amount of at least one derivative as above defined, associated with a pharmaceutical inert vehicle.

Said drugs are administered by the oral, intramuscular and intravenous route.

For oral administration, the drugs are presented in the form of tablets, pills, capsules or drops, patch, spray.

For administration by injection, the drugs are under the form of solution for injection by the intravenous, subcutaneous or intramuscular route produced from sterile or sterilisable solution, or suspension or emulsion.

The invention also relates to the use of said nitrogeneous polycyclic derivatives as chelating agents of transition metals.

Other characteristics and advantages of the invention will be given in the following examples given for illustrative purposes.

Cyclo-Phen preparation:

Bromydrate of 3,8-dihydroxy-1,10-phenanthroline was synthesized through a method optimized in the laboratory (C. Boldron, M. Pitié and B. Meunier, *Synlett.*, 2001, 1629-1631). All the other commercially available reagents and the solvents were used without further purification. The NMR-spectra were recorded on a Bruker 250 MHz apparatus. The mass spectrometer used is a Perkin-Elmer SCIEX API 365 one and the analyses were done in positive mode. The UV-visible spectra were recorded with a Perkin-Elmer Lambda 35 spectrophotometer. Syntheses were monitored by thin-layer silica chromatography (on MERCK 60 F254 TLC aluminium sheets) eluted by CH_2Cl_2 / CH_3OH (9 / 1,

v / v) to which 1 % of concentrated aqueous ammonia (30 %) had been added, and spots were monitored under UV light (violet spots at 254 nm).

Cyclo-Phen synthesis: 2.22 g (6.83 mmol) of cesium carbonate were added to a solution of 0.40 g (1.37 mmol) of 3,8-dihydroxy-1,10-phenanthroline hydrobromide dissolved in 310 mL of anhydrous dimethylsulfoxyde (DMSO). Then a solution of 0.53 g (1.37 mmol) of 1,3-propanediol di-para-tosylate in 80 mL of anhydrous DMSO was added over 1 hour before to heat the mixture 48 hours at 50 °C under nitrogen and vigorous stirring. The volume was reduced to 100 mL then 40 mL of 30 % aqueous ammonia were added and cyclized products were extracted with two volumes of CH_2Cl_2. The organic phase was washed with aqueous ammonia (pH = 10) then evaporated before to be dried under vacuum. A chromatography on silica gel (eluent 1 % triethylamine (TEA) in $CHCl_3$) afforded Cyclo-bi-Phen (31 mg, 0.06 mmol, yield = 9 %) as a white powder. A mixture of Cyclo-tri-Phen and Cyclo-tetra-Phen was then eluted from the column with $CHCl_3$ / TEA / CH_3OH (94 / 5 / 1, v / v / v). After evaporation of the solvent, the two products were dissolved in $CHCl_3$ / CH_3OH (9/3) then Cyclo-tetra-Phen was precipitated by addition of 6 volumes of CH_3OH. The supernatant was evaporated and a flash chromatography on silica gel (eluent 1 % TEA in $CHCl_3$) afforded Cyclo-tri-Phen (14 mg, 0.013 mmol, yield = 3 %) as a white powder. Pure Cyclo-tetra-Phen was obtained from recrystallisation in hot $CHCl_3$ / CH_3OH (3 / 1) as white crystals (10 mg, 0.01 mmol, yield = 3 %).

Cyclo-bi-Phen: ^1H NMR (250 MHz, in $CDCl_3$ / CD_3OD: 3 / 1) δ, ppm: 2.12 (m, 4H), 4.15 (m, 4H), 4.35 (m, 4H), 6.98 (d, ^4J = 3 Hz, 4H), 7.19 (s, 4H), 8.21 (d, ^4J = 3 Hz, 4H). ^{13}C NMR (62.9 MHz in $CDCl_3$ / CD_3OD 3 / 1) δ. ppm: 153.3, 141.9, 138.2, 127.1, 126.6, 115.4, 63.4, 30.4. Mass spectrometry, electrospray, m / z: 505 (MH^+). Elemental analysis: $C_{30}H_{24}N_4O_4 \cdot 0.6\ H_2O$: % theoretical: C 69.92, H 4.93, N 10.87; % found.: C 70.01, H

4.94, N 10.53. UV-vis (H_2O / CH_3OH: 9 / 1): 237 nm (ε = 105000 mol^{-1} cm^{-1}), 281 (29500), 301 (18500), 319 (15000), 338 (9300), 355 (7200).

Cyclo-tri-Phen: ^1H NMR (250 MHz, in $CDCl_3$ / CD_3OD : 3 / 1) δ, ppm: 2.21 (quint, ^3J = 5 Hz, 6H), 4.20 (t, ^3J = 5 Hz, 12H), 7.26 (d, ^4J = 3 Hz, 6H), 7.36 (s, 6H), 8.50 (d, ^4J = 3Hz, 6H). Mass spectrometry, electrospray, m / z: 757 (MH^+). Elemental analysis: $C_{45}H_{36}N_6O_6 \cdot CHCl_3$: % theoretical: C 63.05, H 4.23, N 9.59; % found: C 62.61, H 4.57, N 9.01. UV-vis (H_2O / CH_3OH: 1 / 9): 241 nm (ε = 147000 mol^{-1} cm^{-1}), 280 (44000), 300 (28500), 313 (23000), 339 (11500), 355 (11000).

Cyclo-tetra-Phen: ^1H NMR (250 MHz, in $CDCl_3$ / CD_3OD : 3 / 1) δ, ppm: 2.31 (m, 8H), 4.20 (m, 16H), 7.37 (d, ^4J = 3 Hz, 8H), 7.49 (s, 8H), 8.54 (d, ^4J = 3 Hz, 8H). Mass spectrometry, electrospray, m/z : 1009 (MH^+). Elemental analysis: $C_{60}H_{48}N_8O_8 \cdot 2$ $CHCl_3$: % theoretical: C 59.68, H 4.04, N 8.98; % found: C 59.78, H 3.62, N 8.56. UV-vis (H_2O/CH_3OH: 9 / 1 + 4 HCl): 240 nm (ε = 140000 mol^{-1} cm^{-1}), 283 (53000), 301 (shoulder, 41000), 340 (16000), 356 (14500).

Complexation properties of Cyclo-bi-Phen, Cyclo-tri-Phen and Cyclo-tetra-Phen derivatives in the presence of $CuCl_2$

The complexes were studied by UV -visible spectroscopy and electrospray mass spectrometry.

The formation of a metallic complex resulted in a change of the absorption spectrum of the metallic ion and of the ligand.

Each Cyclo Phen was titrated by $CuCl_2$ to determine the maximal stoechiometry of the Cu complexes which were formed under the experimental conditions.

The studies were carried out between 200 and 420 nm at waves lengths involving the ligand orbitals, The 3 ligands were used in H_2O/MeOH at 10-20 µM. A solution of $CuCl_2$ at 2 mM was used in order to avoid variations of volume of more than 10% the initial volume.

Cyclo bi-Phen was solubilized in methanol/eau: 9/1 at a concentration of 14 µM. The maximal absorption band of the ligand at 237 nm and is submitted to a bathochrome and hypochrome effect during the complexation, a band with a maximal absorption at 345 nm being formed. The complexation with $CuCl_2$ results in the formation of various complexes during the addition of $CuCl_2$.

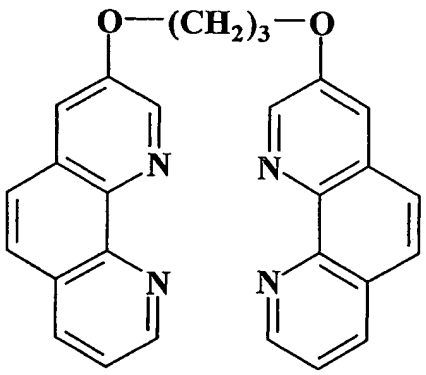
Cyclo-tri-Phen was solubilized in methanol/eau: 9/1 at a concentration of 20µM.5 isobestic points were observed at 227, 248, 283, 297 and 320 nm.

Preliminary toxicity studies on mice with three different chelating agents :

3-Propyl-Clip-Phen (M = 432 Da; preparation according to C. Boldron *et al.*, *Synlett*, 2001, 1629-1631), Cyclo-bi-Phen (M = 504 Da; preparation as described in the present patent application) and Clioquinol (M = 305; 5-chloro-7-iodo-8-hydroxyquinoline, purchased from Sigma).

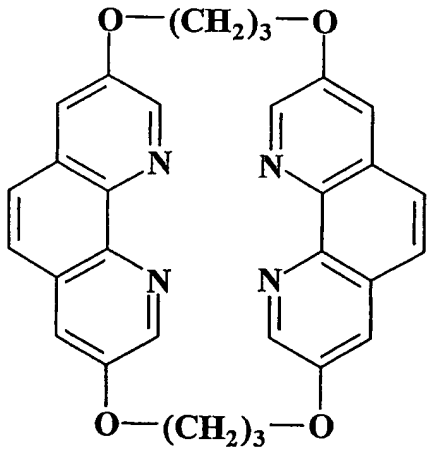
These three compounds were tested on wild-type male FVB mice having a mean weight of 25 grams at 10 mg/kg by intraperitoneal (i.p.) injection at three consecutive days. At day 4, the animals were sacrified and checked for possible anatomical problems. The drugs were initially dissolved in DMSO in the presence of 2.6 equivalents of HCl and then diluted in water.

At 10 mg/kg, all mice survived at day 4 and no anatomical problems have been observed on stomach, spleen, kidneys, liver, heart, lungs and peritoneum.



molecule B = 3-propyl-Clip-Phen

(Phen = ortho-phenanthroline)



molecule G = Cyclo-bi-Phen

Experiments with these three chelating agents with double transgenic mice model of Alzheimer's disease (AD).

Mice over-expressing human APP with the London mutation (V717I) and human PS1 bearing the A242E mutation (APP and PS1

stand for amyloid protein precursor and preseniline 1, respectively) were used. These animals develop many of the the pathological features of AD, including extensive deposition of amyloid plaques, neuritic dystrophy and astroglyosis (animals were identical to that used in the study performed by B. Permanne *et al., FASEB J.*, 2002, vol. 16, 860-862).

Three molecules were evaluated on these double transgenic mice (6-month old) :
3-Propyl-Clip-Phen (molecule B in the histogram below), Cyclo-bi-Phen (molecule G) and Clioquinol (molecule W) (C stands for control, only DMSO diluted in water). Clioquinol has already been used in the treatment AD transgenic mice by Cherny *et al., Neuron,* 2001, vol. 30, 665-676).

The molecules were initially diluted in DMSO in the presence of 2.6 equivalents of HCl and then in water and the animals were treated by i.p. injection with the two Phen derivatives at 5 mg/kg or at 10 mg/kg for Clioquinol, three times per week (monday, wednesday and friday) during 9 consecutive weeks. 9 animals were treated for each drugs (control also included 9 animals). During the 9-week period, one animal was lost in each treatment group and none in the control group.

After 9 weeks of treatment, the animals were sacrified and the amyloid plaque loading brain sections was analyzed by staining with thioflavin S according to the protocol described by K. R. Bales *et al., Nature Genetics*, 1997, vol. 17, 263-264. This method is used to quantify the "old" plaques.

The histogramm below indicate that one Phen derivative, 3-Propyl-Clip-Phen has a negative effect: the plaque loading increased by 16%, whereas Cyclo-bi-Phen is able to reduce the plaque loading by 38%. In the same conditions, the reduction of Clioquinol is only 28%. Taking in consideration, the difference of molecular weight of these two chelators (504 for Cyclo-bi-Phen and 305 for Clioquinol), the 38% reduction has

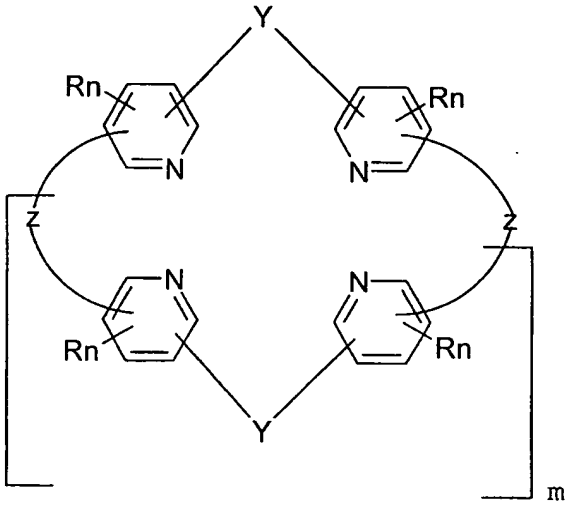
been obtained with 9.9 micromoles/kg with Cyclo-bi-Phen and 32.8 micromoles/kg with Clioquinol, a drug charge 3.3 times higher.

These data obtained on the reduction of thioflavin-S stained amyloid desposit is of particular interest since these thioflavin-staine plaques are now considered as being selectively neurotoxic (see B. Urbanc *et al.*, *PNAS*, 2002, vol. 99, 13990-13995).

This significative reduction of the plaque loading observed with Cyclo-bi-Phen clearly indicate that the Cyclo-Phen derivatives can be considered as drug candidates in the treatment of neurodegenerative diseases where an over-loading of metal ions in brain have been evoked as being one of the main factors of the pathologies such as Alzheimer's disease, Parkinson's disease and any other pathologies related to metal-related misfolding of proteins (Huntington's disease and spongiform encephalopathies).

CLAIMS

1. The use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases, said derivatives having formula (I)



wherein

- Rn is anyone of R1, R2, R3 and R4, which are identical or different and represent H or represent one or several radicals and are selected in the group comprising -OH, an alkyl radical, -O-alkyl group, $-NH_2$,-NH-alkyl, -N (R5, R6), the alkyl being in said radical or groups a C1-C6 alkyl, or an halogen selected between the group consisting of F, Cl, Br,

- Y

- forms a phenyl group with both pyridines, optionally ortho-substituted by a substituent R5, or ortho-disubstituted by R5 and R6, said substituents being identical or different, and selected in the group comprising an alkyl radical, -O-alkyl group, $-NH_2$,-NH-alkyl, -N (R5, R6), the alkyl being in said radical or groups a C1-C6 alkyl, or an halogen selected between the group consisting of F, Cl, Br, or

- represents a group - $(CH_2)_{m1}$-W - $(CH_2)_{m1}$-, with m1 and m2 being 0, 1 or 2, and W being a group $-CH_2-$, -CH (R7), O,

or N (R8, R9), R7, R8 and R9, identical or different, being a C1-C3 alkyl radical, or H,

- Z is a linking arm of formula - A- $(CH_2)_n$-U- $(CH_2)_n$-A-,
  - A being O or NH, and
  - U being selected in the group comprising - $(CH_2)_{n1}$-, - N (R1,R2), -COOH, -OH,

with n being a number from 2 to 6, preferably from 2 to 4, and n1 being 0 or 1,

and the complexes thereof with transition metals, particularly with copper, zinc or iron.

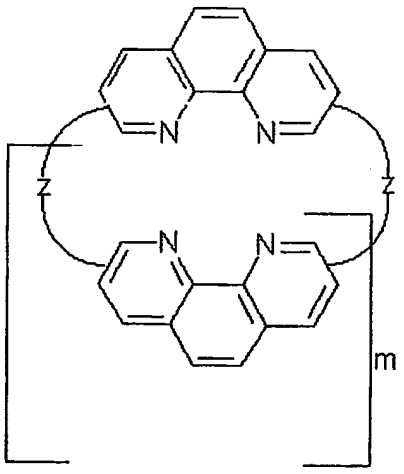
2. The use according to claim 1, wherein said derivatives include 2 cyclic moieties.

3. The use according to claim 1, wherein said derivatives include 3 cyclic moieties.

4. The use according to claim 1, wherein said derivatives include 4 cyclic moieties.

5. The use according to anyone of claims 1 to 4, wherein, in said derivatives, the cyclic moieties consist of Phen moieties.

6. The use according to claim 5, wherein said derivatives are polycyclic Phen having formula (II)



7. The use according to anyone of claims 1 to 6, for treating degenerative diseases comprising Alzheimer, Parkinson, Huntington diseases.

8. The use according to anyone of the preceding claims, wherein the drugs comprise an effective amount of at least one derivative as defined in anyone of claims 1 to 6, associated with a pharmaceutical inert vehicle.

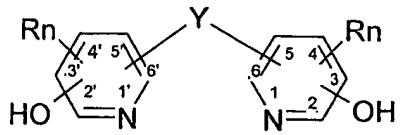
9. The use according to claim 8, wherein the drug is administered by the oral, intramuscular and intravenous route.

10. The use according to claim 9, wherein, for oral administration, the drugs are presented in the form of tablets, pills, capsules or drops, patch, spray.

11. The use according to claim 9, wherein for administration by injection, the drugs are under the form of solution for injection by the intravenous, subcutaneous or intramuscular route produced from sterile or sterilisable solution, or suspension or emulsion.

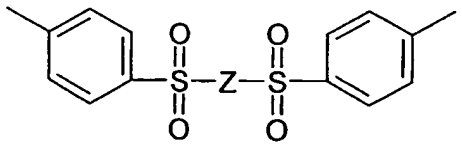
12. A method for preparing the derivatives of anyone of claims 1 to 6, comprising reacting

- a dihydroxy bipyridine derivative of formula (III)



with

- a ditosyl derivative of formula (IV)



wherein Rn, Y and Z are as defined in claim 1.

13. The method of claim 12, wherein the reaction is carried out with high dilution conditions.

14. The method of claim 12 or 13, comprising the use of cesium carbonate.

15. Application of the derivatives defined in anyone of claims 1 to 6 as chelating agents of transition metals.

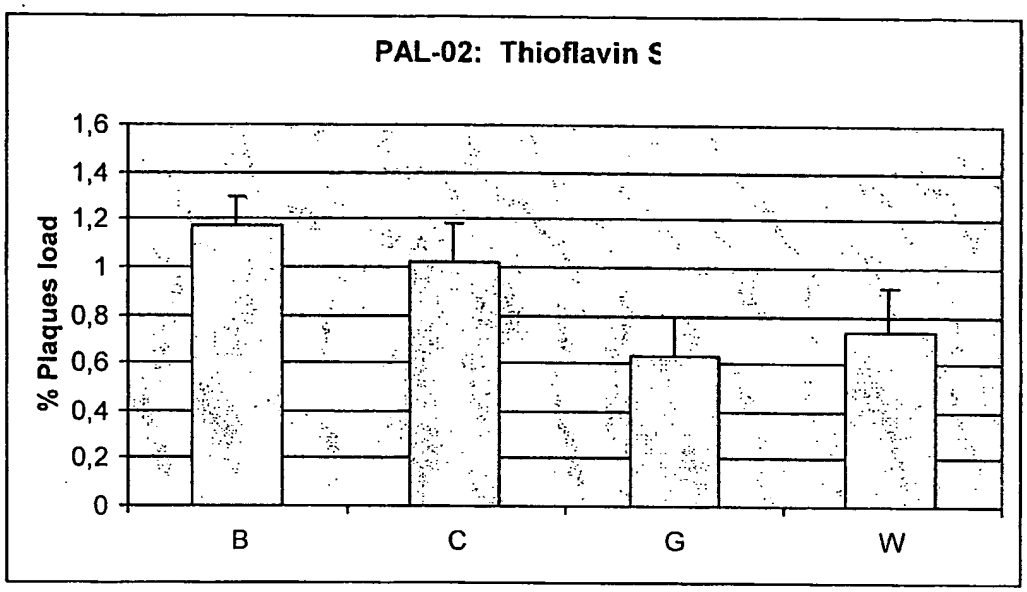


FIGURE 1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicants *(for all designated States except US)*: **PALUMED SA** [FR/FR]; Prologue Biotech, Rue Pierre et Marie Curie, F-31319 LABEGE CEDEX (FR). **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS)** [FR/FR]; 3, Rue Michel-Ange, F-75794 PARIS CEDEX (FR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BOLDRON, Christophe** [FR/FR]; "Tout va bien", 30, Place Sylvain Dumon, F-82400 VALENCE D'AGEN (FR). **PITIE, Marguerite** [FR/FR]; 17, Rue Romain Rolland, F-31520 RAMONVILLE (FR). **MEUNIER, Bernard** [FR/FR]; 7, Impasse des Meuniers, F-31320 CASTENET (FR).

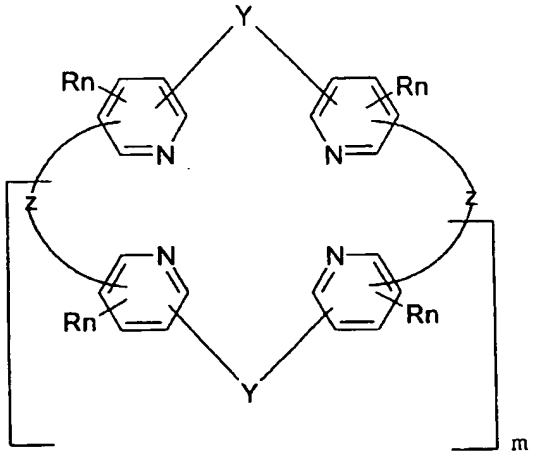
(74) Agents: **PEAUCELLE, Chantal** et al.; Cabinet ARMENGAUD AINE, 3, Avenue Bugeaud, F-75116 PARIS (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK,

[Continued on next page]

(54) Title: NITROGENEOUS POLYCYCLIC DERIVATIVES USEFUL AS CHELATORS OF METAL IONS AND THEIR APPLICATIONS



(I)

(57) Abstract: The invention relates to the use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases, said derivatives having formula : (I) wherein Rn is R1, R2, R3 and R4, identical or different and represent H or one or several radicals selected in the group comprising -OH, alkyl, -O-alkyl, $-NH_2$, -NH-alkyl, -N (R5, R6), the alkyl being a C1-C6 alkyl, or an halogen, - Y forms a phenyl with both pyridines, optionally ortho-substituted by R5, or ortho-disubstituted by R5 and R6, said substitutuents, identical or different, being selected amongst alkyl, -O-alkyl, $-NH_2$, -NH-alkyl, -N (R5, R6), the alkyl being a C1-C6 alkyl, or an halogen, or represents $-(CH_2)_{m1}-W-(CH_2)_{m1}$, with M1 and M2 being 0, 1 or 2, and W being a group $-CH_2-$, -CH-(R7), 0, or N (R8, R9), R7, R8 and R9, identical or different, being a C1-C3 alkyl radical, or H, - Z is $-A-(CH_2)_m-U-(CH_2)_n-A-$, with A = O or N, and U = $-(CH_2)_n-$, -N(R1, R2), -COOH, -OH, with n is 2 to 6, and n1 is 0 or 1, and the complexes thereof with transition metals.

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TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MURALI DORAISWAMY P: "NON-CHOLINERGIC STRATEGIES FOR TREATING AND PREVENTING ALZHEIMER'S DISEASE" CNS DRUGS, ADIS INTERNATIONAL, AUCKLAND, NZ, vol. 16, no. 12, 2002, pages 811-824, XP009033332 ISSN: 1172-7047 page 819-820, paragraph entitled "1.9. Chelation Therapy" ----- -/--	1-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

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17 August 2004	07/09/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Borst, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHERNY R A ET AL: "AQUEOUS DISSOLUTION OF ALZHEIMER'S DISEASE ABETA AMYLOID DEPOSITS BY BIOMETAL DEPLETION" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 274, no. 33, 1999, pages 23223-23228, XP000929630 ISSN: 0021-9258 page 23227-23228, paragraph entitled "Discussion"	1-15
A	CHERNY ROBERT A ET AL: "Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice" NEURON, vol. 30, no. 3, June 2001 (2001-06), pages 665-676, XP002292658 ISSN: 0896-6273 cited in the application page 670-673, paragraph entitled "Discussion"	1-15
A	WO 98/40071 A (GEN HOSPITAL CORP ; BUSH ASHLEY I (US); ATWOOD CRAIG S (US); HUANG XUD) 17 September 1998 (1998-09-17) claim 8	1-15
A	BOLDRON C ET AL: "Simple and efficient syntheses of 1,10-phenanthrolines substituted at C3 or C3 and C8 by methoxy or hydroxy groups" SYNLETT 2001 GERMANY, no. 10, 2001, pages 1629-1631, XP001183054 ISSN: 0936-5214 cited in the application figure and scheme 2	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2004/004016

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9840071 A	17-09-1998	AU 748768 B2	13-06-2002
		AU 6548498 A	29-09-1998
		CA 2284170 A1	17-09-1998
		EP 1007048 A1	14-06-2000
		JP 2001514661 T	11-09-2001
		WO 9840071 A1	17-09-1998

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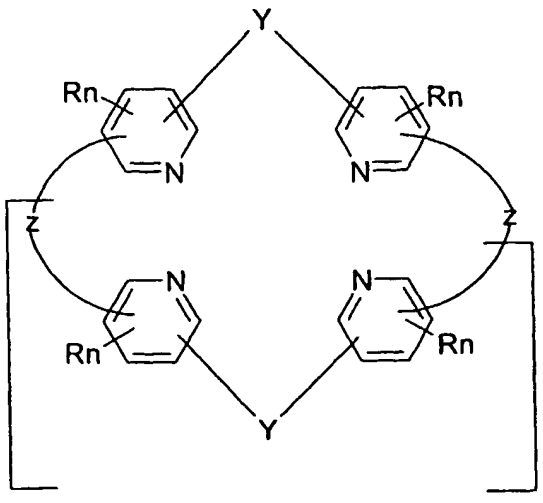
Published:
— *with international search report*

[Continued on next page]

(54) Title: NITROGENEOUS POLYCYCLIC DERIVATIVES USEFUL AS CHELATORS OF METAL IONS AND THEIR APPLICATIONS



(I)

(57) Abstract: The invention relates to the use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases, said derivatives having formula : (I) wherein Rn is R1, R2, R3 and R4, identical or different and represent H or one or several radicals selected in the group comprising -OH, alkyl, -O-alkyl, $-NH_2$, -NH-alkyl, -N (R5, R6), the alkyl being a C1-C6 alkyl, or an halogen, - Y forms a phenyl with both pyridines, optionally ortho-substituted by R5, or ortho-disubstituted by R5 and R6, said substitutuents, identical or different, being selected amongst alkyl, -O-alkyl, $-NH_2$, -NH-alkyl, -N (R5, R6), the alkyl being a C1-C6 alkyl, or an halogen, or represents $-(CH_2)_{m1}-W-(CH_2)_{m1}$, with M1 and M2 being 0, 1 or 2, and W being a group $-CH_2-$, -CH-(R7), 0, or N (R8, R9), R7, R8 and R9, identical or different, being a C1-C3 alkyl radical, or H, - Z is $-A-(CH_2)_m-U-(CH_2)_n-A-$, with A = O or N, and U = $-(CH_2)_n-$, -N(R1, R2), -COOH, -OH, with n is 2 to 6, and n1 is 0 or 1, and the complexes thereof with transition metals.

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"Nitrogeneous polycyclic derivatives useful as chelators of metal ions and their applications"

The invention relates to the use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases. Said derivatives are useful as ligands to form complexes with transition metals, and the invention also relates to the use of such derivatives containing ligands as active principles.

Many studies have recently shown the major role of metal ions (copper, zinc, iron, ...) in modification of the folding or the aggregation of proteins, leading then to serious pathologies. Several neurodegenerative diseases (Alzheimer's disease, Parkinson and Huntington diseases, spongiform encephalopathies, ...) involve these disastrous non-desired interactions between metal ions and proteins.

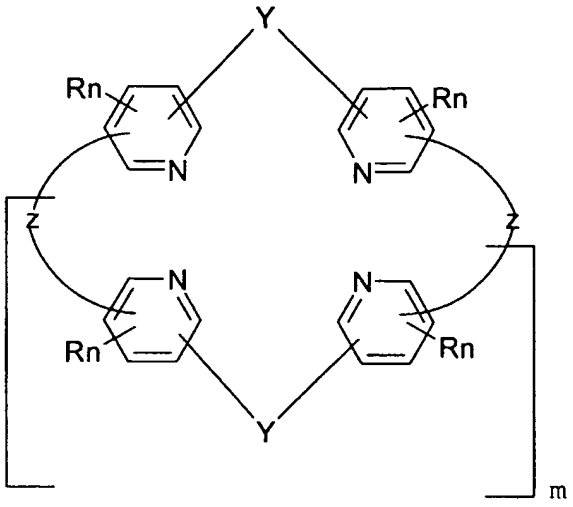
In the case of Alzheimer disease, the pathology is associated with the aggregation of □-type amyloid peptides in the brain, leading to the formation of amyloid plaques. The accumulation of redox active metal ions in these amyloid plaques is deemed to be responsible for oxidative stress inducing neuronal lesions in the brain which result in irreversible loss of intellectual faculties.

The use of a ligand of metal ions like Clioquinol led to improvements in Alzheimer's disease indicating that therapeutic approaches are possible with metal ion chelators in neurodegeneratives diseases.

Recent works of the inventors on phenanthroline derivatives ("Phen" will be used to designate 1,10-phenanthroline) has demonstrated the benefit of complexing copper with two phenanthroline ligands connected to each other. It was therefore decided to prepare new cyclic uncharged ligands called "Cyclo-Phen", small and sufficiently hydrophobic to be able to cross the barriers (first the

intestinal barrier and then the blood brain barrier to go to coordinate the metal ions (copper in preference) which are present in excess in the pathogen proteins.

The invention thus relates to the use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases, said derivatives having formula (I)



wherein

- Rn is anyone of R1, R2, R3 and R4, which are identical or different and represent H or represent one or several radicals and are selected in the group comprising -OH, an alkyl radical, -O-alkyl group, $-NH_2$,-NH-alkyl, -N (R5, R6), the alkyl being in said radical or groups a C1-C6 alkyl, or an halogen selected between the group consisting of F, Cl, Br,

- Y

- forms a phenyl group with both pyridines, optionally ortho-substituted by a substituent R5, or ortho-disubstituted by R5 and R6, said substituents being identical or different, and selected in the group comprising an alkyl radical, -O-alkyl group, $-NH_2$,-NH-alkyl, -N (R5, R6), the alkyl being in said radical or groups a C1-C6 alkyl, or an halogen selected between the group consisting of F, Cl, Br, or

- represents a group - $(CH_2)_{m1}$-W - $(CH_2)_{m1}$-, with m1 and m2 being 0, 1 or 2, and W being a group $-CH_2-$, -CH (R7), O, or N (R8, R9), R7, R8 and R9, identical or different, being a C1-C3 alkyl radical, or H,

- Z is a linking arm of formula - A- $(CH_2)_n$-U- $(CH_2)_n$-A-,

- A being O or NH, and

- U being selected in the group comprising - $(CH_2)_{n1}$-, - N (R1,R2), -COOH, -OH,

with n being a number from 2 to 6, preferably from 2 to 4, and n1 being 0 or 1,

and the complexes thereof with transition metals, particularly with copper, zinc or iron.

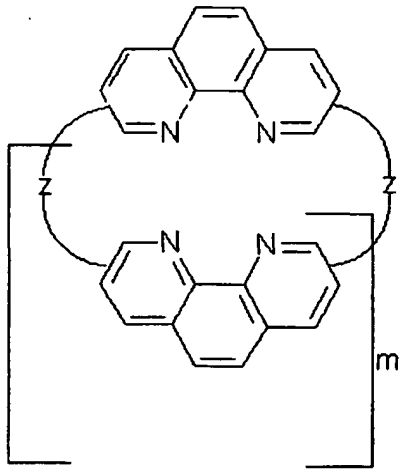
According to an embodiment of the invention, said derivatives include 2 cyclic moieties.

According to another embodiment of the invention, said derivatives include 3 cyclic moieties.

According to still another embodiment, said derivatives include 4 cyclic moieties.

Preferably, the cyclic moieties consist of Phen moieties.

The invention particularly relates to the use of polycyclic Phen derivatives having formula (II)

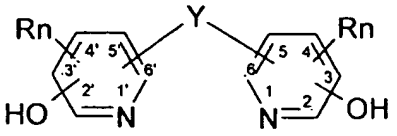


The invention particularly relates to the use of derivatives having 2, 3 or 4 Phen moieties,

The invention also relates to a method for the preparation of said derivatives.

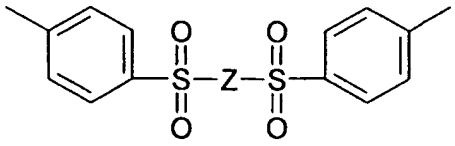
The method of the invention comprises reacting

- a dihydroxy bipyridine derivative of formula (III)



with

- a ditosyl derivative of formula (IV)



wherein Rn, Y and Z are as above defined.

The reaction is carried out with high dilution conditions to limit oligomerizations.

The precursor of formula (III) is preferably used at concentrations of 0.1 to 20 mM in a polar solvent, such as DMSO.

In order to avoid β-elimination reactions, a weak base like cesium carbonate is used.

The derivatives of the invention have a low molecular weight (MW of 504 for the cyclic bi-Phen) and are poorly charged. Therefore they are able to cross the blood brain barrier in both directions (the metal ions present in excess in the pathogen proteins have to be chelated and the resulting complex has to be exported towards the blood circulation conducting to its ultimate excretion),

Their structure can be altered to adjust the chelation selectivity in order to target certain metal ions.

It results from the pharmacological studies carried out with said derivatives that they have new activity spectrum and

are particularly appropriate for the treatment of neurodegenerative diseases as above mentioned.

The invention relates to the use of said derivatives for preparing drugs for treating degenerative diseases comprising Alzheimer, Parkinson, Huntington diseases.

Said drugs comprise an effective amount of at least one derivative as above defined, associated with a pharmaceutical inert vehicle.

Said drugs are administered by the oral, intramuscular and intravenous route.

For oral administration, the drugs are presented in the form of tablets, pills, capsules or drops, patch, spray.

For administration by injection, the drugs are under the form of solution for injection by the intravenous, subcutaneous or intramuscular route produced from sterile or sterilisable solution, or suspension or emulsion.

The invention also relates to the use of said nitrogeneous polycyclic derivatives as chelating agents of transition metals.

Other characteristics and advantages of the invention will be given in the following examples given for illustrative purposes.

Cyclo-Phen preparation:

Bromydrate of 3,8-dihydroxy-1,10-phenanthroline was synthesized through a method optimized in the laboratory (C. Boldron, M. Pitié and B. Meunier, *Synlett.*, 2001, 1629-1631). All the other commercially available reagents and the solvents were used without further purification. The NMR-spectra were recorded on a Bruker 250 MHz apparatus. The mass spectrometer used is a Perkin-Elmer SCIEX API 365 one and the analyses were done in positive mode. The UV-visible spectra were recorded with a Perkin-Elmer Lambda 35 spectrophotometer. Syntheses were monitored by thin-layer silica chromatography (on MERCK 60 F254 TLC aluminium sheets) eluted by CH_2Cl_2 / CH_3OH (9 / 1,

v / v) to which 1 % of concentrated aqueous ammonia (30 %) had been added, and spots were monitored under UV light (violet spots at 254 nm).

Cyclo-Phen synthesis: 2.22 g (6.83 mmol) of cesium carbonate were added to a solution of 0.40 g (1.37 mmol) of 3,8-dihydroxy-1,10-phenanthroline hydrobromide dissolved in 310 mL of anhydrous dimethylsulfoxyde (DMSO). Then a solution of 0.53 g (1.37 mmol) of 1,3-propanediol di-para-tosylate in 80 mL of anhydrous DMSO was added over 1 hour before to heat the mixture 48 hours at 50 °C under nitrogen and vigorous stirring. The volume was reduced to 100 mL then 40 mL of 30 % aqueous ammonia were added and cyclized products were extracted with two volumes of CH_2Cl_2. The organic phase was washed with aqueous ammonia (pH = 10) then evaporated before to be dried under vacuum. A chromatography on silica gel (eluent 1 % triethylamine (TEA) in $CHCl_3$) afforded Cyclo-bi-Phen (31 mg, 0.06 mmol, yield = 9 %) as a white powder. A mixture of Cyclo-tri-Phen and Cyclo-tetra-Phen was then eluted from the column with $CHCl_3$ / TEA / CH_3OH (94 / 5 / 1, v / v / v). After evaporation of the solvent, the two products were dissolved in $CHCl_3$ / CH_3OH (9/3) then Cyclo-tetra-Phen was precipitated by addition of 6 volumes of CH_3OH. The supernatant was evaporated and a flash chromatography on silica gel (eluent 1 % TEA in $CHCl_3$) afforded Cyclo-tri-Phen (14 mg, 0.013 mmol, yield = 3 %) as a white powder. Pure Cyclo-tetra-Phen was obtained from recrystallisation in hot $CHCl_3$ / CH_3OH (3 / 1) as white crystals (10 mg, 0.01 mmol, yield = 3 %).

Cyclo-bi-Phen: ^1H NMR (250 MHz, in $CDCl_3$ / CD_3OD: 3 / 1) δ, ppm: 2.12 (m, 4H), 4.15 (m, 4H), 4.35 (m, 4H), 6.98 (d, ^4J = 3 Hz, 4H), 7.19 (s, 4H), 8.21 (d, ^4J = 3 Hz, 4H). ^{13}C NMR (62.9 MHz in $CDCl_3$ / CD_3OD 3 / 1) δ. ppm: 153.3, 141.9, 138.2, 127.1, 126.6, 115.4, 63.4, 30.4. Mass spectrometry, electrospray, m / z: 505 (MH^+). Elemental analysis: $C_{30}H_{24}N_4O_4 \cdot 0.6\ H_2O$: % theoretical: C 69.92, H 4.93, N 10.87; % found.: C 70.01, H

4.94, N 10.53. UV-vis (H_2O / CH_3OH: 9 / 1): 237 nm (ε = 105000 mol^{-1} cm^{-1}), 281 (29500), 301 (18500), 319 (15000), 338 (9300), 355 (7200).

Cyclo-tri-Phen: ^1H NMR (250 MHz, in $CDCl_3$ / CD_3OD : 3 / 1) δ, ppm: 2.21 (quint, ^3J = 5 Hz, 6H), 4.20 (t, ^3J = 5 Hz, 12H), 7.26 (d, ^4J = 3 Hz, 6H), 7.36 (s, 6H), 8.50 (d, ^4J = 3Hz, 6H). Mass spectrometry, electrospray, m / z: 757 (MH^+). Elemental analysis: $C_{45}H_{36}N_6O_6 \cdot CHCl_3$: % theoretical: C 63.05, H 4.23, N 9.59; % found: C 62.61, H 4.57, N 9.01. UV-vis (H_2O / CH_3OH: 1 / 9): 241 nm (ε = 147000 mol^{-1} cm^{-1}), 280 (44000), 300 (28500), 313 (23000), 339 (11500), 355 (11000).

Cyclo-tetra-Phen: ^1H NMR (250 MHz, in $CDCl_3$ / CD_3OD : 3 / 1) δ, ppm: 2.31 (m, 8H), 4.20 (m, 16H), 7.37 (d, ^4J = 3 Hz, 8H), 7.49 (s, 8H), 8.54 (d, ^4J = 3 Hz, 8H). Mass spectrometry, electrospray, m/z : 1009 (MH^+). Elemental analysis: $C_{60}H_{48}N_8O_8 \cdot 2\ CHCl_3$: % theoretical: C 59.68, H 4.04, N 8.98; % found: C 59.78, H 3.62, N 8.56. UV-vis (H_2O/CH_3OH: 9 / 1 + 4 HCl): 240 nm (ε = 140000 mol^{-1} cm^{-1}), 283 (53000), 301 (shoulder, 41000), 340 (16000), 356 (14500).

Complexation properties of Cyclo-bi-Phen, Cyclo-tri-Phen and Cyclo-tetra-Phen derivatives in the presence of $CuCl_2$

The complexes were studied by UV -visible spectroscopy and electrospray mass spectrometry.

The formation of a metallic complex resulted in a change of the absorption spectrum of the metallic ion and of the ligand.

Each Cyclo Phen was titrated by $CuCl_2$ to determine the maximal stoechiometry of the Cu complexes which were formed under the experimental conditions.

The studies were carried out between 200 and 420 nm at waves lengths involving the ligand orbitals, The 3 ligands were used in H_2O/MeOH at 10-20 µM. A solution of $CuCl_2$ at 2 mM was used in order to avoid variations of volume of more than 10% the initial volume.

Cyclo bi-Phen was solubilized in methanol/eau: 9/1 at a concentration of 14 µM. The maximal absorption band of the ligand at 237 nm and is submitted to a bathochrome and hypochrome effect during the complexation, a band with a maximal absorption at 345 nm being formed. The complexation with $CuCl_2$ results in the formation of various complexes during the addition of $CuCl_2$.

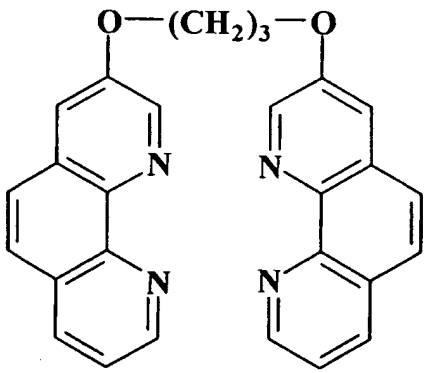
Cyclo-tri-Phen was solubilized in methanol/eau: 9/1 at a concentration of 20µM.5 isobestic points were observed at 227, 248, 283, 297 and 320 nm.

Preliminary toxicity studies on mice with three different chelating agents :

3-Propyl-Clip-Phen (M = 432 Da; preparation according to C. Boldron *et al., Synlett*, 2001, 1629-1631), Cyclo-bi-Phen (M = 504 Da; preparation as described in the present patent application) and Clioquinol (M = 305; 5-chloro-7-iodo-8-hydroxyquinoline, purchased from Sigma).

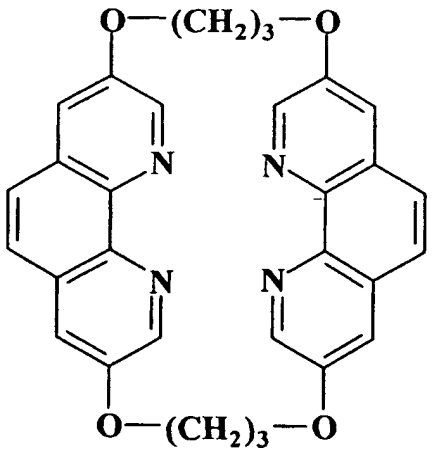
These three compounds were tested on wild-type male FVB mice having a mean weight of 25 grams at 10 mg/kg by intraperitoneal (i.p.) injection at three consecutive days. At day 4, the animals were sacrified and checked for possible anatomical problems. The drugs were initially dissolved in DMSO in the presence of 2.6 equivalents of HCl and then diluted in water.

At 10 mg/kg, all mice survived at day 4 and no anatomical problems have been observed on stomach, spleen, kidneys, liver, heart, lungs and peritoneum.



molecule B = 3-propyl-Clip-Phen

(Phen = ortho-phenanthroline)



molecule G = Cyclo-bi-Phen

Experiments with these three chelating agents with double transgenic mice model of Alzheimer's disease (AD).

Mice over-expressing human APP with the London mutation (V717I) and human PS1 bearing the A242E mutation (APP and PS1

stand for amyloid protein precursor and preseniline 1, respectively) were used. These animals develop many of the the pathological features of AD, including extensive deposition of amyloid plaques, neuritic dystrophy and astroglyosis (animals were identical to that used in the study performed by B. Permanne *et al., FASEB J.,* 2002, vol. 16, 860-862).

Three molecules were evaluated on these double transgenic mice (6-month old) :

3-Propyl-Clip-Phen (molecule B in the histogram below), Cyclo-bi-Phen (molecule G) and Clioquinol (molecule W) (C stands for control, only DMSO diluted in water). Clioquinol has already been used in the treatment AD transgenic mice by Cherny *et al., Neuron,* 2001, vol. 30, 665-676).

The molecules were initially diluted in DMSO in the presence of 2.6 equivalents of HCl and then in water and the animals were treated by i.p. injection with the two Phen derivatives at 5 mg/kg or at 10 mg/kg for Clioquinol, three times per week (monday, wednesday and friday) during 9 consecutive weeks. 9 animals were treated for each drugs (control also included 9 animals). During the 9-week period, one animal was lost in each treatment group and none in the control group.

After 9 weeks of treatment, the animals were sacrified and the amyloid plaque loading brain sections was analyzed by staining with thioflavin S according to the protocol described by K. R. Bales *et al., Nature Genetics,* 1997, vol. 17, 263-264. This method is used to quantify the "old" plaques.

The histogramm below indicate that one Phen derivative, 3-Propyl-Clip-Phen has a negative effect: the plaque loading increased by 16%, whereas Cyclo-bi-Phen is able to reduce the plaque loading by 38%. In the same conditions, the reduction of Clioquinol is only 28%. Taking in consideration, the difference of molecular weight of these two chelators (504 for Cyclo-bi-Phen and 305 for Clioquinol), the 38% reduction has

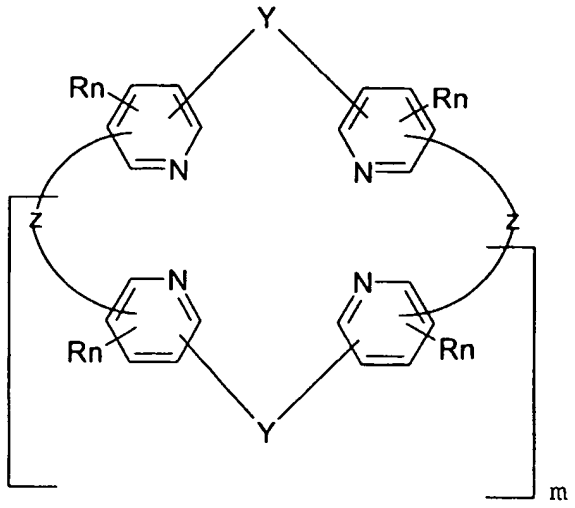
been obtained with 9.9 micromoles/kg with Cyclo-bi-Phen and 32.8 micromoles/kg with Clioquinol, a drug charge 3.3 times higher.

These data obtained on the reduction of thioflavin-S stained amyloid desposit is of particular interest since these thioflavin-staine plaques are now considered as being selectively neurotoxic (see B. Urbanc *et al., PNAS*, 2002, vol. 99, 13990-13995).

This significative reduction of the plaque loading observed with Cyclo-bi-Phen clearly indicate that the Cyclo-Phen derivatives can be considered as drug candidates in the treatment of neurodegenerative diseases where an over-loading of metal ions in brain have been evoked as being one of the main factors of the pathologies such as Alzheimer's disease, Parkinson's disease and any other pathologies related to metal-related misfolding of proteins (Huntington's disease and spongiform encephalopathies).

CLAIMS

1. The use of nitrogeneous polycyclic derivatives for preparing drugs for treating neurodegenerative diseases, said derivatives having formula (I)



wherein

- Rn is anyone of R1, R2, R3 and R4, which are identical or different and represent H or represent one or several radicals and are selected in the group comprising -OH, an alkyl radical, -O-alkyl group, $-NH_2$,-NH-alkyl, -N (R5, R6), the alkyl being in said radical or groups a C1-C6 alkyl, or an halogen selected between the group consisting of F, Cl, Br,

- Y

- forms a phenyl group with both pyridines, optionally ortho-substituted by a substituent R5, or ortho-disubstituted by R5 and R6, said substituents being identical or different, and selected in the group comprising an alkyl radical, -O-alkyl group, $-NH_2$,-NH-alkyl, -N (R5, R6), the alkyl being in said radical or groups a C1-C6 alkyl, or an halogen selected between the group consisting of F, Cl, Br, or

- represents a group - $(CH_2)_{m1}$-W - $(CH_2)_{m1}$-, with m1 and m2 being 0, 1 or 2, and W being a group $-CH_2-$, -CH (R7), O,

or N (R8, R9), R7, R8 and R9, identical or different, being a C1-C3 alkyl radical, or H,

- Z is a linking arm of formula - A- $(CH_2)_n$-U- $(CH_2)_n$-A-,
  - A being O or NH, and
  - U being selected in the group comprising - $(CH_2)_{n1}$-, - N (R1,R2), -COOH, -OH,

with n being a number from 2 to 6, preferably from 2 to 4, and n1 being 0 or 1,

and the complexes thereof with transition metals, particularly with copper, zinc or iron.

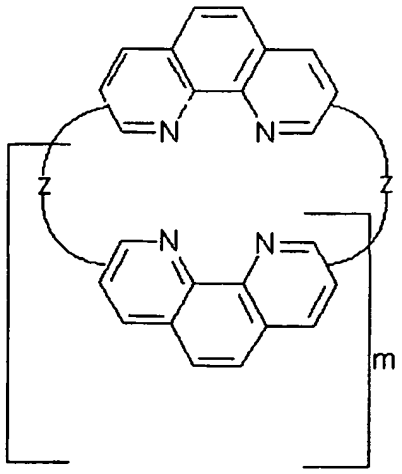
2. The use according to claim 1, wherein said derivatives include 2 cyclic moieties.

3. The use according to claim 1, wherein said derivatives include 3 cyclic moieties.

4. The use according to claim 1, wherein said derivatives include 4 cyclic moieties.

5. The use according to anyone of claims 1 to 4, wherein, in said derivatives, the cyclic moieties consist of Phen moieties.

6. The use according to claim 5, wherein said derivatives are polycyclic Phen having formula (II)



7. The use according to anyone of claims 1 to 6, for treating degenerative diseases comprising Alzheimer, Parkinson, Huntington diseases.

8. The use according to anyone of the preceding claims, wherein the drugs comprise an effective amount of at least one derivative as defined in anyone of claims 1 to 6, associated with a pharmaceutical inert vehicle.

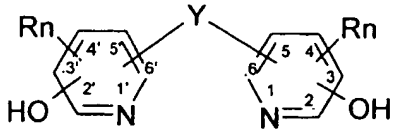
9. The use according to claim 8, wherein the drug is administered by the oral, intramuscular and intravenous route.

10. The use according to claim 9, wherein, for oral administration, the drugs are presented in the form of tablets, pills, capsules or drops, patch, spray.

11. The use according to claim 9, wherein for administration by injection, the drugs are under the form of solution for injection by the intravenous, subcutaneous or intramuscular route produced from sterile or sterilisable solution, or suspension or emulsion.

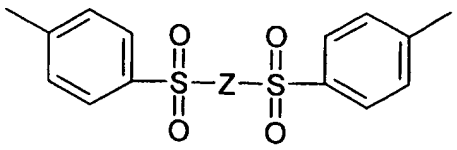
12. A method for preparing the derivatives of anyone of claims 1 to 6, comprising reacting

- a dihydroxy bipyridine derivative of formula (III)



with

- a ditosyl derivative of formula (IV)



wherein Rn, Y and Z are as defined in claim 1.

13. The method of claim 12, wherein the reaction is carried out with high dilution conditions.

14. The method of claim 12 or 13, comprising the use of cesium carbonate.

15. Application of the derivatives defined in anyone of claims 1 to 6 as chelating agents of transition metals.

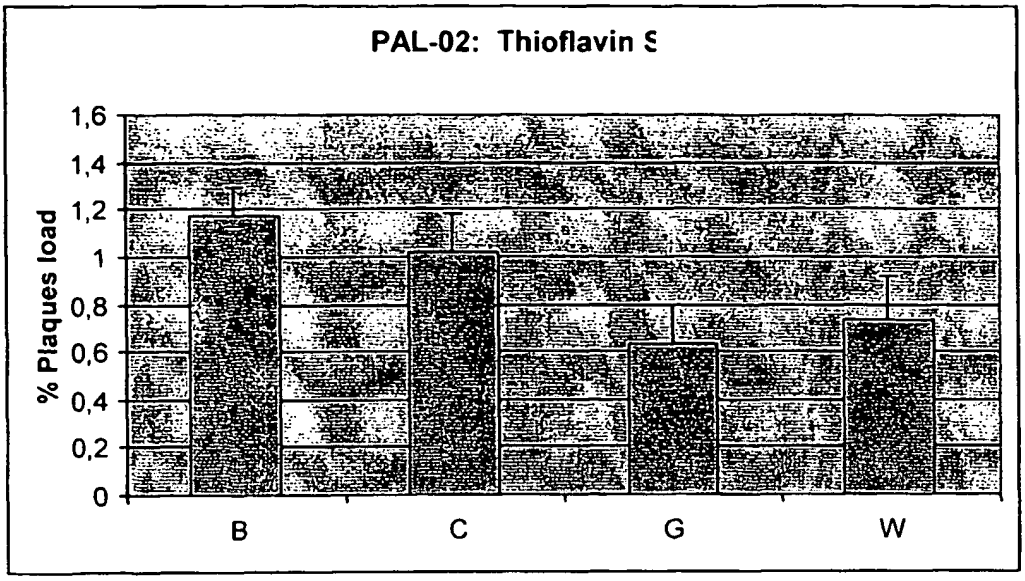


FIGURE 1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/004016

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D498/22 A61K31/4745 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MURALI DORAISWAMY P: "NON-CHOLINERGIC STRATEGIES FOR TREATING AND PREVENTING ALZHEIMER'S DISEASE" CNS DRUGS, ADIS INTERNATIONAL, AUCKLAND, NZ, vol. 16, no. 12, 2002, pages 811-824, XP009033332 ISSN: 1172-7047 page 819-820, paragraph entitled "1.9. Chelation Therapy" ----- -/--	1-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 2004	07/09/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Borst, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/004016

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHERNY R A ET AL: "AQUEOUS DISSOLUTION OF ALZHEIMER'S DISEASE ABETA AMYLOID DEPOSITS BY BIOMETAL DEPLETION" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 274, no. 33, 1999, pages 23223-23228, XP000929630 ISSN: 0021-9258 page 23227-23228, paragraph entitled "Discussion"	1-15
A	CHERNY ROBERT A ET AL: "Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice" NEURON, vol. 30, no. 3, June 2001 (2001-06), pages 665-676, XP002292658 ISSN: 0896-6273 cited in the application page 670-673, paragraph entitled "Discussion"	1-15
A	WO 98/40071 A (GEN HOSPITAL CORP ; BUSH ASHLEY I (US); ATWOOD CRAIG S (US); HUANG XUD) 17 September 1998 (1998-09-17) claim 8	1-15
A	BOLDRON C ET AL: "Simple and efficient syntheses of 1,10-phenanthrolines substituted at C3 or C3 and C8 by methoxy or hydroxy groups" SYNLETT 2001 GERMANY, no. 10, 2001, pages 1629-1631, XP001183054 ISSN: 0936-5214 cited in the application figure and scheme 2	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2004/004016

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9840071 A	17-09-1998	AU 748768 B2	13-06-2002
		AU 6548498 A	29-09-1998
		CA 2284170 A1	17-09-1998
		EP 1007048 A1	14-06-2000
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		WO 9840071 A1	17-09-1998